

**What is claimed:**

1. A process for enhanced secretion of a polypeptide in bacteria, comprising:
  - 5 (a) culturing bacterial cells that contain a recombinant expression vector comprising a first DNA sequence encoding a polypeptide that can be secreted by the bacteria and a second DNA sequence encoding a charged, amino-acid tag covalently bonded at the carboxy-terminus of said polypeptide, such that the polypeptide is produced by the cells; and
  - 10 (b) optionally, recovering the polypeptide from the culture medium.
2. The process of claim 1, wherein said tag comprises one or more charged amino acid residues.
- 15 3. The process of claim 2, wherein said tag comprises at least two negatively charged amino acid residues or at least two positively charged amino acid residues.
4. The process of claim 3, wherein said tag comprises two negatively  
20 charged amino acid residues, selected from the group consisting of D and E.
5. The process of claim 4, wherein said tag comprises two D residues.
6. The process of claim 3, wherein said tag comprises two positively charged  
25 amino acid residues, selected from the group consisting of K and N.
7. The process of claim 6, wherein said tag comprises two K residues.
8. The process of claim 1, wherein said bacteria is a *Bacillus* species.
- 30 9. The process of claim 8, wherein said bacteria is *B. subtilis*.

10. The process of claim 1, wherein said expression vector further includes a DNA sequence encoding a signal peptide operatively linked to said first DNA sequence.

5 11. The process of claim 10, wherein said signal peptide is *B. licheniformis*  $\alpha$ -amylase (AmyL) signal peptide.

10 12. The process of claim 1, wherein said polypeptide is a heterologous protein selected from the group consisting of hormones, enzymes, and growth factors.

13. The process of claim 12, wherein said protein is human interleukin.

15 14. A method for enhancing the secretion of a heterologous polypeptide in a *Bacillus* species, comprising: substituting one or more of the C-terminal amino acids residues of said polypeptide with at least one charged amino acid residue, or adding one or more charged amino acid residues to the C-terminus of said polypeptide.

20 15. The method of claim 14, wherein the last two amino acid residues of said polypeptide are substituted with a D.

16. The method of claim 14, wherein the last two amino acid residues of said polypeptide are substituted with a E.

25 17. The method of claim 14, wherein the last two amino acid residues of said polypeptide are substituted with a K.

30 18. The method of claim 14, wherein the last two amino acid residues of said polypeptide are substituted with a N.

19. The method of claim 14, wherein two D residues are added at the C-terminus of said polypeptide.

5 20. The method of claim 14, wherein two E residues are added at the C-terminus of said polypeptide.

21. The method of claim 14, wherein two K residues are added at the C-terminus of said polypeptide.

10 22. The method of claim 14, wherein two N residues are added at the C-terminus of said polypeptide.

23. A method of reducing the susceptibility of a polypeptide to an extracellular protease of a microorganism, said method comprising  
15 substituting one or more of the C-terminal amino acids residues of said polypeptide with at least one charged amino acid residue, or adding one or more charged amino acid residues to the C-terminus of said polypeptide.

24. An expression cassette comprising a first DNA sequence encoding a  
20 protein of interest and a second DNA sequence encoding a tag, wherein the tag is covalently attached to the C-termini of the protein of interest when transcribed.

25 25. The expression cassette of claim 24 further comprising a third DNA sequence encoding a signal sequence.

26. The expression cassette of claim 25 wherein the signal sequence is for the sec-dependent secretory pathway.

27. The expression cassette of claim 26 wherein the signal sequence is AprE.

28. The expression cassette of claim 26 wherein the signal sequence is the  
5 *B. licheniformis*  $\alpha$ -amylase (AmyL) signal peptide.

29. The expression cassette of claim 25 wherein the signal sequence is for the Twin Arginine Translocation secretory pathway.

10 30. A recombinant protein of interest comprising a protein of interest covalently attached at its C-termini to a tag.

31. The recombinant protein of interest of Claim 29 wherein said tag is at least one amino acid residue wherein said residue is a charged residue.

15 32. The recombinant protein of interest of Claim 30 wherein said charged residue is negatively charged.

33. The recombinant protein of interest of Claim 31 wherein said negatively  
20 charged residue is D.

34. The recombinant protein of interest of Claim 31 wherein said negatively charged residue is E.

25 35. The recombinant protein of interest of Claim 30 wherein said charged residue is positively charged.

36. The recombinant protein of interest of Claim 34 wherein said positively charged residue is K.

37. The recombinant protein of interest of Claim 34 wherein said positively charged residue is N.

38. The recombinant protein of interest of Claim 29 wherein said tag is selected from the group comprising SsrA<sup>NN</sup> (SEQ ID NO:\_\_\_), SsrA<sup>DD</sup> (SEQ ID NO:\_\_\_), SsrA<sup>KK</sup> (SEQ ID NO:\_\_\_), and SsrA<sup>EE</sup> (SEQ ID NO:\_\_\_).

39. A chimeric polypeptide comprising (i) a secretion signal peptide, (ii) a heterologous polypeptide and (iii) a tag sequence.

40. The chimeric polypeptide of Claim 39 wherein the secretion signal peptide is selected from sec-dependent or tat-dependent secretion signals.

41. The chimeric polypeptide of Claim 40 wherein the secretion signal peptide is a tat-dependent secretion signal.

42. The chimeric polypeptide of Claim 41 wherein the secretion signal peptide is selected from PhoD or LipA derived from *Bacillus*.

43. The chimeric polypeptide of Claim 40 wherein the secretion signal peptide is a sec-dependent secretion signal.

44. The chimeric polypeptide of Claim 43 wherein the secretion signal peptide is selected from AmyL or AprE secretion signal peptides.

45. A nucleic acid molecule comprising a first nucleotide sequence encoding a signal sequence operatively linked to a second nucleotide sequence encoding a heterologous polypeptide wherein the last two codons of the polypeptide have been replaced with codons for a charged amino acid residue.

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FOOTNOTES 10001-2254660

46. The nucleic acid molecule of claim 45 wherein the charged amino acid residue is positively charged.

47. The nucleic acid molecule of claim 46 wherein the charged amino acid  
5 residue is K.

48. The nucleic acid molecule of claim 46 wherein the charged amino acid residue is N.

10 49. The nucleic acid molecule of claim 45 wherein the charged amino acid residue is negatively charged.

50. The nucleic acid molecule of claim 49 wherein the charged amino acid residue is D.

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51. The nucleic acid molecule of claim 49 wherein the charged amino acid residue is E.

52. A nucleic acid molecule comprising a first nucleotide sequence encoding  
20 a signal sequence operatively linked to a second nucleotide sequence  
encoding a heterologous polypeptide and a third nucleotide sequence  
encoding a tag sequence.